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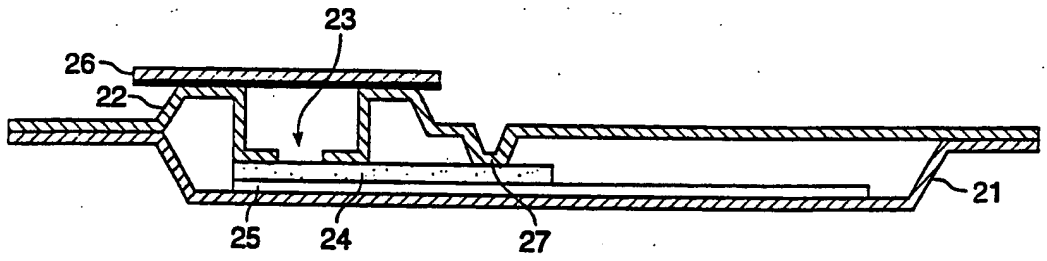

(57) Abstract

The present invention describes a test device for detecting or measuring analytes in a test sample, which comprises a transparent, impervious, rigid and hollow housing containing an assay test material. The device is constructed such that light can pass through both the housing and assay material. Various assays can be conducted using the device, resulting in an optical change or changes usually based upon the amount of analyte in the test sample, which change is detected visually or by an optical instrument.

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TRANSPARENT ASSAY TEST DEVICES AND METHODS

BACKGROUND OF THE INVENTION

The invention disclosed concerns novel test devices for use in conducting diagnostic assays to determine the presence or absence of an analyte in a test sample. Typically, the test device comprises a transparent or translucent housing or casing and contains a chromatic assay. Light is then transmitted through the housing such that the transmitted or absorbed light is detectable by visual inspection or machine measurement at the end of the assay and the change in the light intensity or pattern is correlated to the presence or absence of the analyte in the sample.

Diagnostic or immunological assays or tests, depending on the recognition and measurement of antigens and/or antibodies in body tissues and fluids of patients, are widely used as an aid in clinical analysis. The immunological tests that are in routine use depend on well-established principles of antigen-antibody recognition and the formation of immune complexes.

Specific immunoassays have been developed which comprise, in part, the utilization of porous solid supports for antigens or immunoglobulins where one of the reactants is immobilized on the supports. The immunologic activity or binding reactions are usually restricted to a particular test zone or area on the support through the use of immobilization. Oftentimes, multiple analytes can be detected on the supports at the completion of the assay.

An inherent difficulty in the preexisting diagnostic tests is that false positive or false negative responses, unrelated to the particular immunologic analysis, may result as a consequence of the inability of the interpreter of the assay to differentiate between a true response and false response associated with proteins bound up in the reaction site nonspecifically or associated with a failure or error in the

assay procedure. For example, when as in a competitive assay, a reduction in signal at the test zone is indicative of the presence of an analyte in a test sample, it is often difficult to interpret a partial reduction in the signal in those assays using an optical change. As a result, subjective visual analysis can sometimes cause an incorrect reading due to the inherent difficulties in distinguishing the correct response caused by the subtle change in the optical properties.

In some of the existing devices, the results cannot be read properly due to the configuration of the device, which hampers the visibility of the assay results being determined. Typically, the assay is conducted on a test strip format within an opaque housing, which impairs light access to the strip. Other times, the test strip is completely removed from its packaging, which can result in errors and contamination in the assay.

Although some tests contain a clear or open window on the top of the device, they do not allow the operator or interpreter to fully see the change in optical properties at the end of the assay. Therefore, the results can be read incorrectly. Furthermore, the open window exposes the test strip and reagents to the elements, contaminating the test strip and compromising the assay. Although some of the devices are further sealed in foil packages until use to protect the test strip, they require additional materials and add bulkiness to the package, which means an increase in the manufacturing and shipment costs, as well as additional storage costs.

Typically, the existing tests can only be read from one side of the device, limiting the optical instrumentation that can be used. It would be preferable if the test device could be read by a variety of instruments as well as an individual to improve the accuracy of the reading.

SUMMARY OF THE INVENTION

The present invention is an improvement over the current assay devices used for interpretation of assay results and will reduce or obviate the subjectivity associated with the existing systems. It comprises a transparent or translucent,

impervious, rigid or semi-rigid, and hollow housing or means which is sealed. Within the housing is contained an assay material with one or more assay reagents located at predetermined, selected, or fixed sites.

A substantial amount of light can be transmitted through the assay material and housing of the device such that optical changes as a result of the assay can be detected visually or by optical instruments. The light can be transmitted from any side of the housing including the back of the device. This enables the operator to backlight the device, which is an advantage in certain assay formats and improves the detection of the analyte through the change in optical properties. Having the light transmitted through the entire device allows the operator or instrument to read the depth of the assay material. Therefore, what is embedded in the material can be detected as well as what is on its surface and the optical change can include a change in absorption as well as in wavelength or intensity.

Since the device is completely sealed before use, it can be shipped without the need for an external pouch, with or without desiccants or absorbents, used to protect the assay material and reagents from damage or contamination. In addition, the reagents, including those that might be considered hazardous, are contained within the sealed housing, minimizing or eliminating exposure to the operator or the handler. The device also is easy to use, since all reagents, except the test sample, are contained within the device and the results can be read from any direction. Furthermore, once the assay is completed, the device can be resealed for disposal or storage, again minimizing or eliminating the contact between hazardous or infectious substances and the assay operator.

Once the assay is to be run, the device can be opened, thereby exposing an area or portion of the assay material. The material can be dipped in, immersed in, contacted with, or subjected to, the test sample. Once an adequate amount of the sample necessary to conduct the assay is on the material, the test sample can be removed. In some cases, the test sample can remain in contact with the assay

material without adversely affecting the performance of the assay.

Any assay can be run so long as it results in an optical change which correlates to the presence or absence of the analyte or analytes in the test sample. Once the assay is complete, the change can be measured visually or by an optical instrument.

The invention also comprises diagnostic kits wherein the test device and the ancillary reagents necessary to conduct the assay of interest are part of the kit. Ancillary reagents can include buffers, diluents, standards, and the like. In most cases, the kits are complete except for the addition of the test sample.

DESCRIPTION OF THE FIGURES

The figures described below are selected examples of the various aspects of this invention, but should not be construed as being limiting. In Figure 1, there is shown a test device wherein the hollow housing (1) encompasses an assay material in the form of a test strip (2). The test strip (2) is comprised of various regions. The first region is at the open or exposed distal end of the housing and is the sample receiving area (3), wherein the test sample is contacted with the area to begin the assay. The receiving area also contains a color indicator or mobile label reagent, such as a colloidal particle. The sample moves up the test strip by capillary action to subsequent regions. Therefore, for this particular device, the material which comprises the test strip has a capillary action which is greater than the gravitational pull on the liquid. The device can be immersed in varying levels of test sample without compromising the assay, since the air in the device prevents excess sample from entering. The sample fluid migrates through the assay material by capillary action and displaces the air trapped in the housing.

Certain mobile reagents or immobile reagents are found in one or more of these subsequent regions (4), called test reaction zones. It is in one or more of these zones that the analyte in the sample will be complexed to the test strip

and then the amount complexed can be measured by optical changes. At the end of the test strip is a region (5), called the terminal absorbent end, which consists of an absorbent material used to retain some or all of the excess test sample and/or test reagents that have moved through the test strip. In Figure 1, the test device is placed in a container (6) which holds the test sample of the patient (7). The protrusion at the distal end of the housing (8) protects the bottom of the test strip from contact with the bottom of the container (6).

Figure 2 is a cross-section view of the device shown in Figure 1 before it is opened at the distal end. Therefore, the device is completely sealed at both ends. The device consists of a hollow housing (1) which encloses an assay material in the form of a test strip (2). The test strip (2) is comprised of various regions, including the sample receiving area (3) and the test reaction zones (4). A blocking material (10), which also can have light transmitted through it, is next to, or abuts, the assay material. The device can be cut or snapped open at the scored area (9) on the housing before beginning the assay. The scored area is angled such that when the device is opened, the protrusion at the distal end of the housing (8) and the sample receiving area (3) are exposed.

In Figure 3, there is shown an alternative test device, wherein the hollow housing (21) encompasses an assay material (not shown). The upper surface of the housing is raised (22) to form a well (23), which receives the test sample.

Figure 4 is a cross-section view of a device similar to Figure 3 with a foil material, which can be peeled back (26), covering the well (23). The housing (21) contains the sample receiving pad (24) in contact with the assay material (25). The raised upper surface of the housing (22) forms the well (23) for sample addition. The upper surface of the housing (27) is notched and acts as a pressure bar, holding the sample receiving pad and assay material in place.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is an improvement over current assay materials and devices used for the interpretation of assay results and reduces or obviates the subjectivity associated with many of these existing assays. The invention expands the visual and instrumental reading options for the assay measurement and for the interpretation of the results.

The test device comprises a sealed housing, which is opened just prior to conducting the assay. The housing is constructed of a moisture impervious material, which is substantially transparent or translucent. Lodged within the housing is an assay format, preferably on one or more test strips or disks, using a porous material or filter capable of transporting the test sample and appropriate assay reagents by capillary action. The assay material has an area for receiving the test sample, preferably an absorbent sample receiving area, which contains, communicates with, or is in contact with, the test sample during the assay run. Various test zones are on the assay material, which have various capture agents or control agents for conducting the assay. The assay reagents and presence of analyte in the sample must result in a change in the optical properties of selected reagents on, or in, the assay material at the end of the assay. Once the assay is completed, the results can be read by the operator visually or with the aid of an optical instrument.

Housing

The housing of the device has the following characteristics. It is made from a translucent or transparent material, such that a substantial amount of light can be transmitted through the material and used to measure or detect the presence or absence of an analyte contained in a test sample as indicated by the assay. The test device housing is constructed from a rigid material, which can be molded or shaped such that the center of the device is hollow and is sealed, and which material is impervious to moisture, liquid and other contaminants. Therefore, the housing is made of any impervious or non-porous solid material that can retain a rigid

shape and will not be damaged during routine shipment of the device. The housing also can be made from various materials, such as clear plastics or glass, with plastic preferred, such as vinyls and acrylics. Although the housing can be molded or constructed in any size or shape, so long as the assay can be conducted and the results read optically, it is preferred that the housing be made such that no additional hermetic packaging is necessary. For example, the device's housing can be shipped without excess foil packaging, which is costly and bulky. Furthermore, in a preferred embodiment of this invention, the housing can be oval or rectangular for easy stacking and storing.

The shape and construction of the housing should not interfere with, or hamper, the conduct of the assay test and its results. Therefore, the capillary action of the test must not be impaired. In addition, the casing or housing is capable of allowing the unhindered transmission of light through all or part of it, usually in a predetermined location or locations on the assay material. This location contains the response being detected or measured by the assay and correlates to the presence or absence of the test sample analyte.

In some preferred embodiments, the housing will be shaped to form a pressure bar holding the assay material in place. In addition, parts of the housing can be raised to form a well for the test sample addition.

Although the test device is hermetically sealed during transport and storage, one end or area of the device must be opened in order to conduct the assay. Therefore, just prior to the start of the assay, the end or area must be exposed for sample addition. Preferably, the material covering or encompassing the end or area is structurally part of the housing, which is scored for easy breakage by the operator. In other words, the housing is sealed until the assay operator snaps off one end and exposes the sample receiving area. After the assay is run, the end which was snapped off can be placed back on the end of the housing or discarded. For example, the sample receiving area can be covered by a resealable cap or cover. Alternatively, and also preferred, foil or a clear

adhesive can be used which can be removed or peeled back and then placed over the area at the completion the assay. Covering the sample receiving area at the end of the assay will prevent contamination of the results and improve handling and disposal of the device, especially when the sample area contains infectious or other undesirable materials.

Assay Test Material

The assay material must be of a sufficient size to adequately and accurately conduct the selected assay. Furthermore, there must be enough room within the housing for the assay material to expand upon addition of the test sample. The assay material also is secured within the housing such that the securing means or supports do not hamper or interfere with the assay or the optical reading upon its completion. The housing should not interfere with the action of the assay by inhibiting the capillary movement of the sample or assay reagents.

Since the results of the assay are read optically, light or various wavelengths of light must pass through the assay material. Therefore, the porous filter, membrane, matrix, or assay support and any necessary backing material utilized in the invention is made of any suitable inert material which transmits light. The assay material should not dissolve the reactants or components of the assay and it should have negligible non-specific attraction for these components. However, it must be able to act as a reaction surface for the interaction of the capture agent and analyte, such that the capture agent and analyte can be immobilized. Therefore, although it is porous, it must be able to retain or bind the required components for the assay through physical or chemical means. For example, the capture agent can be coupled to the assay material through chemical bonds or reactions; or the capture agent can be trapped, adhered or adsorbed on a particle, for example, a latex particle; which particle is supported, bound, or trapped on or within the material.

The assay material can be a matrix or solid support usually composed of a mat of compressed fibers, such as a mat

of glass or synthetic fibers or a porous paper mat. It may also be constructed of other porous materials known to those skilled in the art, such as sintered glass, synthetic polymers, gels, mixtures of these substances, and the like, with the preferred material being nylon or nitrocellulose. Nylon 66 cast on a non-woven polyester support with either a co-cast resin containing quartonary ammonium groups or carboxyl groups available from Pall Corporation, Glen Cove, NY is a preferred material. Also preferred is nitrocellulose available from Gelman Corporation. The total amount of test sample which traverses the test reaction zones can be controlled by varying the absorbance area capability, varying the material used, or by limiting the sample applied.

The assay material can be constructed in a variety of shapes, such as strips, films, sheets, plates, dipsticks, and the like, depending upon the particular assay format desired. Preferably, the material is in the form of a strip. The material is from about 100 to about 200 microns (μ) in thickness and its pore size is from about 5.0 to about 12.0 μ. The preferred thickness is about 150 μ with a pore size of 8.0 μ. Where the capture agent is coupled to a particle, such as a latex particle, which is retained, or immobilized, on or within the material, it is required that the pore size be sufficient to prevent those particles from being passed completely through, but allows the sample and other test reactants to permeate.

Test Reaction Zone

The test reaction or test zone is defined as a finite region or site on, or in, the assay material wherein the capture agent and analyte and other reactants in the assay can interact, complex, react, or bind by physical or chemical means. The capture agent can be immobilized in the test zone for interaction with the analyte from the test sample. One skilled in the art will appreciate that the assay material can contain one or multiple, or a plurality of, reaction test zones and can range in size and shape, such as a bar, a plus sign, a

circle, a checkmark, and the like. One or more zones can be used as positive and/or negative controls for the assay.

Test Sample

The test sample or specimen for use in the assay can be obtained from a variety of sources, preferably from animals in the form of biological samples. Most preferred is a test sample from a human patient or host which is in the form of a liquid, semi-liquid, or fluid, such as whole or fractionated blood, urine, sputum, tears, tissue extracts, and the like. The preferred source of the human sample is plasma, serum, or urine. In addition, the apparatus of this invention can be used to detect analytes in chemical or environmental test samples, such as particular organic chemicals. The test sample can be applied to, or brought in contact with, the device of this invention, or the device can be inserted or dipped into the sample. The amount of sample must be sufficient for completion of the assay, but not an excess amount that will compromise the performance of the test. The sample can also be diluted, concentrated, prefiltered, or pretreated before use in the assay.

Analyte

The analyte or analytes to be detected, measured, or determined from the test sample can be a variety of items, such as proteins; peptides; antigens; antibodies; fragments of antibodies, such as the Fc and Fab regions, variable and constant regions, and the heavy and light chains; immunoglobulins; nucleic acid oligomers, such as RNA or DNA; drugs or pharmacological agents; hormones; vitamins; extracts from parasites, allergens, bacteria, viruses, or virus particles; metabolites; organic or chemical compounds; toxins; and the like. (See US Patent 4,690,907.) The present invention can be used to detect one or multiple analytes in an assay by using one or more test reaction zones and/or one or more assay materials. Preferred analytes for use in this invention include:

a. Proteins, such as allergens, protein extracts of pollens, foods, and animal danders;
b. Human plasma proteins, such as albumin, microalbumin, and creatinine kinase;
c. Immunoglobulins, such as IgG, IgA, IgM, and IgE;
d. Protein hormones, such as human chorionic gonadotropin (HCG), luteinizing hormone (LH), and parathyroid hormone;
e. Haptens;
f. Steroids, such as estrogen and digoxin;
g. Therapeutic drugs, such as antibiotics and prostaglandins;
h. Drugs of abuse, such as benzoylecgonine (BE), opiates, and cannabinols;
i. Infectious disease agents, such as bacterial agents Streptococcus, Chlamydia, Rubella, Helicobacter, viral agents, Hepatitis viruses, human immunodeficiency virus (HIV), Herpes viruses, fungal agents, Candida, and Aspergillus; and
j. Cancer markers, carcinoembryonic antigen (CEA), prostate specific antigen (PSA), alpha fetoprotein (AFP), and the like.

Most preferred analytes include HCG, LH, Rubella, and Streptococcus types A and B.

Capture Agent

The capture agent is any receptor, complexing agent, binding partner, binding reagent, or reacting agent or is any substance which reacts with, binds to, complexes with, or interacts with, the analyte, such that the analyte is bound, fixed, or immobilized by its interaction with the capture agent. For example, when the analyte being detected is an antigen, the capture agent can be a polyclonal or monoclonal antibody which binds to the analyte. A small amount of the binding reagent or capture agent is dropped, added, coupled, sprayed, or placed onto the assay material at each area designated as a test reaction zone for that capture agent. The amount of agent required will vary according to the material

porosity and the structure and thickness of the test reaction zone. It is important, especially where there is a single piece of material in the device with two or more test reaction zones contained therein, that the capture agent or binding reagent does not migrate to, or contaminate, another test reaction zone, which may contain a different capture agent for use in the assay. It is also important the capture agent adheres, adsorbs, covalently binds, complexes, or is otherwise immobilized on, or within, the test reaction zone for a period of time sufficient to carry out the desired assay.

The concentration of the agent will vary according to the analyte being detected and the assay method and capture agent being utilized. Each test reaction or control zone can be treated with a blocking reagent, which may be bovine serum albumin, milk casein, or other blocking reagent used in conventional techniques, to negate any undesirable reactive sites remaining on the assay material in the test reaction or control zone.

Color Indicator or Mobile Label Reagent

An indicator (usually an antigen or antibody) capable of specifically participating in, or with, the analyte-capture agent binding complex reaction, may be labeled by a variety of means. For the purposes of this invention, the preferred indicator label results in an optical or color change, or color formation in the test or control zone; therefore, the indicator is called the color indicator. Conventional color labels include chromophores, fluorophores, enzymes, dyes, colored or colorable particles or combinations thereof. The color labels to which the indicator may be bound are usually termed direct labels and indirect labels.

The direct labels are those which are immediately visible to the naked eye or to an instrument capable of detecting or measuring such labels, such as a spectrophotometer, fluorometer, and the like. These direct labels include, but are not limited to,:

a. colored or colorable particles, such as latex particles of various colors, sizes, and chemical

characteristics, examples of which are given in US Patent 4,837,168;

b. gold sol labels, which are available commercially from Sigma Chemical Co., St. Louis, MO as monodispensed colloidal particles, in size ranging from about 5 to about 80 nanometers (nm), which readily and passively adsorb the assay reagents without additional chemical or physical modification or processes; or particles, such as silver, copper, zinc, and ferric colloids;

c. fluorescent labels, such as fluorescein or phycoerythrin; and

d. insoluble dye particles, which may be selectively sized by centrifugation and coupled to the reagent by passive absorption at an acid pH, followed by backcoating with bovine serum albumin and which may be contained in vesicles or liposomes, examples of which are given in U.S. Patent 4,703,017.

Preferred direct labels for use in this invention are intensely colored latex particles available from Molecular Probes, Eugene, OR or Interfacial Dynamics Corporation, Portland, OR with diameters from about 0.01 microns (μ) to about 0.05 μ, which particles adsorb the analyte without chemical modification. Also preferred, are colloidal particles or dyes.

The indirect labels include, but are not limited to, enzymes coupled or conjugated to the analyte or other indicator reactants in the assay. The preferred enzymes are alkaline phosphatase and horse radish peroxidase. These enzymes react with a substrate to cause the color change, such as para-nitrophenylphosphate (p-NPP) or bromocresol indoxyl phosphate/nitroblue tetrazolium (BCIP/NBT) for alkaline phosphatase; or hydrogen peroxide, o-phenylenediamine (OPD), tetramethylbenzidine (TMB), or 2,2'-azino-di(3-ethylbenzthiazolinesulfonic acid (ABTS) for horse radish peroxidase. Regardless of which enzyme and substrate are chosen, pH should be optimized for proper color development.

For example, the targeted antigen or antibody may thereby be directly or indirectly measured or visualized at the test zone by the incorporation of the color indicator in the test zone site. This incorporation of the color indicator can occur prior to, simultaneous with, or after, the addition of the analyte to the test reaction zone. The intensity of color of the indicator complexed on the exposed test reaction area or zone, after removal of unbound reagents, is indicative of the presence or absence of the analyte in the sample being assayed.

Immunological Binding Assays

There are several well recognized immunological specific binding assays to determine the presence and concentration of particular substances, such as enzyme immunoassays (EIA). (See for example, U.S. Patents 4,366,241; 4,376,110; 4,517,288; and 4,837,168.) [See also Basic and Clinical Immunology 7th Edition (D. Stites and A. Terr ed.) 1991.] The immunological assays generally fall into two categories. In the competitive binding assay, the sample analyte competes with a labeled analyte, the indicator, for specific binding sites on the binding reagent. The concentration of labeled analyte bound to the capture agent is inversely proportional to the amount of free analyte present in the sample. In the second, the sandwich assay, the sample analyte is bound between two analyte-specific binding reagents, one of which is labeled, the indicator, in order to measure or detect the resultant complex by visual or instrument means. Commonly, the two specific binding reagents or capture agents are both antibodies with specificities for different epitopes of the antigen analyte and an unlabeled first antibody is immobilized on a solid support or carrier.

In an alternative sandwich assay, the first capture agent may be distinctly different from the second labeled binding reactant, the indicator. In this approach the immobilized capture agent may be an antigen, i.e. a bacterial or viral substance, or allergen, which binds an immunoglobulin or antibody in the sample. The labeled reactant, the indicator, will then be directed towards the sample

immunoglobulin complexed with the immobilized first reagent. In these sandwich assays, the amount of bound labeled reactant, the indicator, is directly proportional to the concentration of bound sample antibody. In some sandwich assays, the two binding reactants may be two monoclonal antibodies, or one monoclonal and one polyclonal. (See US Patent 4,376,110.)

These solid phase immunoassay procedures can employ inert porous assay materials, where one of the antigen or antibody partners is immobilized within a finite and regulated test reaction zone on the assay material, which antigen or antibody is capable of specifically binding and/or immobilizing the targeted analyte of the reaction. The bound partner is not directly measurable and therefore techniques using color indicators can be employed to facilitate indirect visual or instrumental measurement of the targeted analyte captured at the test reaction zone.

The assay run time will depend upon the assay format, analyte, volume of solution, concentrations, and the like. Usually, the assays will be carried out at ambient temperature, although they can be conducted over a range of temperatures, such as 15 to 30°C. It is a preferred embodiment of this invention that wash steps be eliminated.

Preferably, the assay material within the housing is constructed such that a sample receiving area is at one end of the material, followed by various test reaction zones. For example, the sample receiving area is next to a first test reaction zone which contains at a predetermined location: a colloidal particle labeled reagent, a chromatic, fluorescent, or chemiluminescent labeled capture agent or binding reagent. This labeling reagent is mobile and alternatively, can be located within the sample receiving area. As the analyte moves by capillary action through the test reaction zones, it will come into contact with other binding reagents. Within the test reaction zones will be specific agents usually permanently immobilized within the assay material to complex with the analyte and labeled binding reagent. These zones contain the results which are measured by the change in the light intensity or light pattern. At the terminal end of the assay material,

there will be another absorbent area, which will receive the mobile reagents and excess fluid from the test sample after they move through the test reaction zones.

In addition, there can be transparent or translucent colored reference zones within or on the assay material, which can be near or next to the test reaction zone. These colored reference zones can be used in the assay, such that the optical change in the test zones can be compared to the colored reference zones.

Visual or Optical Measurements

The results of the assay and therefore, the detection or the measurement of the analyte's presence in the test sample, can be read visually by the assay operator or by an optical instrument. Visual interpretations can be enhanced by shining a backlight through the housing and thereby through the assay material to improve the optical determination. Other optically detectable changes can be measured, such as a change in the light intensity in the test reaction zone of interest or a change in the light reflected back from the reaction zone, which changes are dependent upon the amount of the analyte complexed within the zone.

In the cases where there is a spectrophotometric measurement, ultraviolet rays or infrared rays can be utilized. In fluorophotometric systems, the excitation and emission wavelength can be selected accordingly to maximize the optical measurement.

All publications and other references or patent documents herein are incorporated by reference. It is to be understood that the above description is intended to be illustrative and not restrictive. Many embodiments will be apparent to those of skill in the art upon reviewing the above description. The scope of the invention should, therefore, should be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled.

WHAT IS CLAIMED IS:

1. A test device comprising:

a. a transparent, impervious and rigid housing, which is sealed;

b. an assay material contained within the housing; and

c. one or more reagents located at predetermined sites on the assay material;

whereby a substantial amount of light can be transmitted through the material and the housing.

2. The test device of Claim 1 wherein one area of the housing can be opened, exposing a portion of the assay material.

3. A test device as shown in Figure 2.

4. A test device as shown in Figure 4.

5. A method for detecting the presence of an analyte in a test sample comprising the steps of:

a. opening the one area of the test device of Claim 2 and exposing a portion of the assay material;

b. placing the test sample in contact with the exposed portion;

c. conducting an assay, which results in an optical change on, or in, the assay material correlating with the presence of analyte; and

d. determining the optical change by visual or optical instrument means.

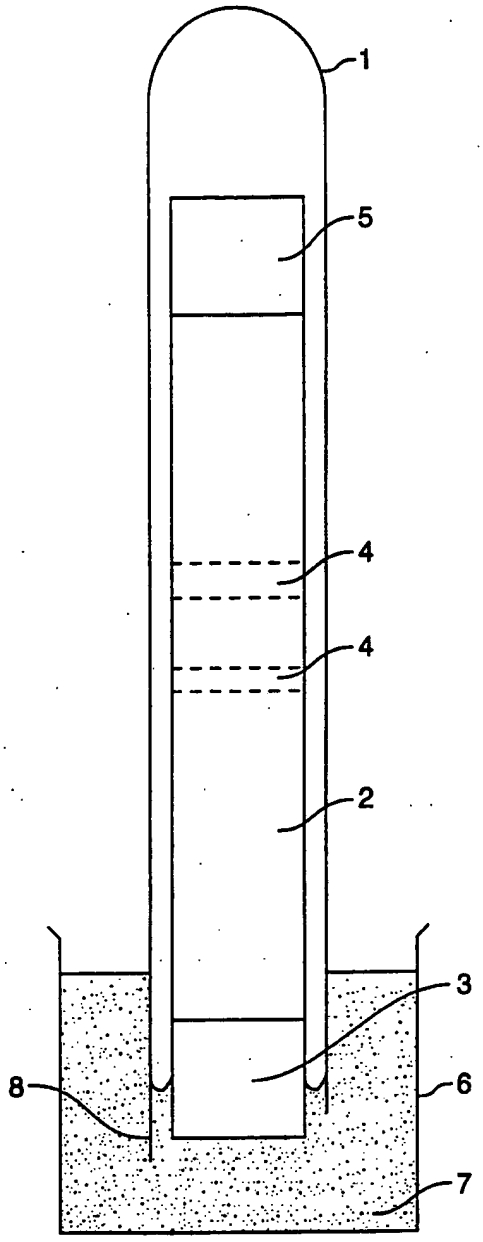

FIG. 1

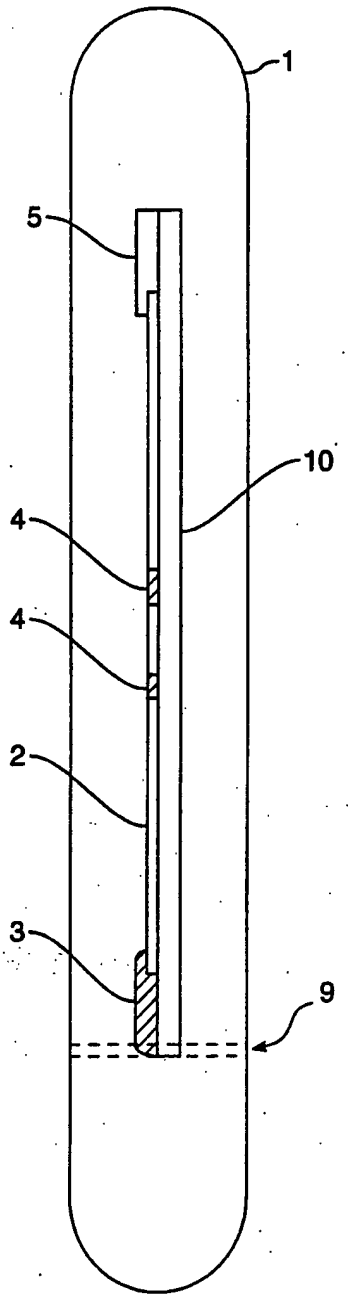

FIG. 2

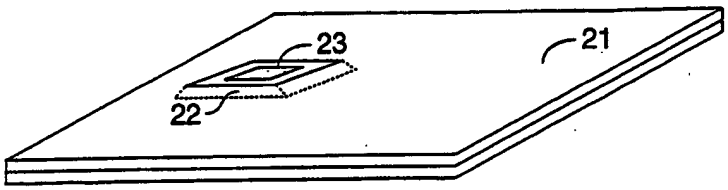

FIG. 3

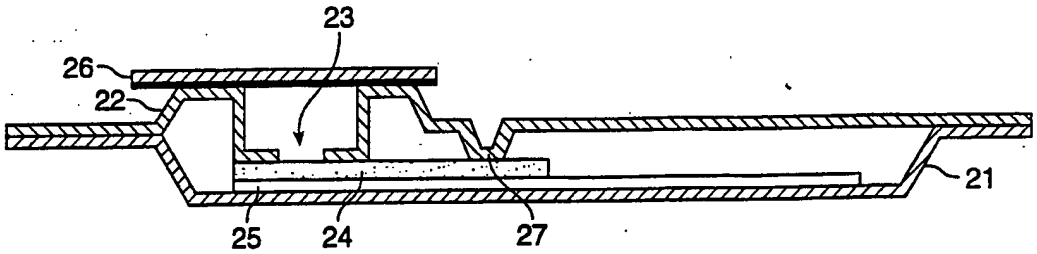

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/06709

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 G01N33/53; G01N33/543; G01N33/52

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	G01N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	US,A,4 189 304 (E.C.ADAMS ET AL.) 19 February 1980 see column 2; claims; figure 5	1,2,5
Y	EP,A,0 226 767 (BAYER AG.) 1 July 1987 see column 1	1,2,5
Y	EP,A,0 296 724 (QUIDEL) 28 December 1988 see page 8 - page 9; figures	1,2,5
A	EP,A,0 432 631 (BOEHRINGER MANNHEIM GMBH) 19 June 1991 see page 2 - page 4; figure 2	1,4,5
A	DE,A,2 711 201 (A.SUEDHOFF ET AL.) 21 September 1978 see page 13 - page 17; figures	1,2,3,5

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
04 OCTOBER 1993	13. 10. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	HITCHEN C.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	EP,A,0 189 925 (GENETIC DIAGNOSTIC CORPORATION) 6 August 1986 see page 5 - page 6; figures	1,2,3,5

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9306709
SA 76704

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 04/10/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4189304	19-02-80	None	
EP-A-0226767	01-07-87	DE-A- 3540526	27-05-87
		DE-A- 3686759	22-10-92
		JP-A- 62119454	30-05-87
		US-A- 4824640	25-04-89
EP-A-0296724	28-12-88	JP-A- 1059069	06-03-89
		US-A- 4943522	24-07-90
EP-A-0432631	19-06-91	DE-A- 3941150	20-06-91
		JP-A- 3279861	11-12-91
DE-A-2711201	21-09-78	None	
EP-A-0189925	06-08-86	AU-A- 5280086	07-08-86
		JP-A- 61223561	04-10-86

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